

METHOD FOR DETECTING THE EXPRESSION OF AN ENVELOPE
PROTEIN OF A HUMAN ENDOGENOUS RETROVIRUS AND USES OF
A GENE CODING FOR SAID PROTEIN

5 Retroviruses are enveloped viruses which bear glycoprotein spicules encoded by the viruses, at their surface. These envelope glycoproteins are synthesized in the form of polyprotein precursors (Pre-env) which are then cleaved by cellular proteases into mature
10 surface (SU) protein and into transmembrane (TM) protein. Envelope glycoproteins are involved in the entry of viruses into host cells. They specifically recognize and bind to cell surface receptors and are necessary for the fusion of the viral envelope and the
15 cell membranes of the host. The receptor and the envelope are multimeric or oligomeric molecules. For all enveloped viruses, the interactions of the envelope glycoproteins with the cellular receptor(s) lead to conformational rearrangements of the envelope required
20 for exposure of the fusion peptide. The fusion takes place at the surface of the cell or in cellular vesicles, depending on the pathway of endocytosis of the virion. In addition, in order to allow entry of the virus, fusion mediated by the viral surface proteins
25 may, under certain conditions, cause cell-to-cell fusion, resulting in the formation of giant multinucleated cells or syncytia. The formation of syncytia takes place via at least two pathways: a virion may simultaneously fuse with two cells, in which
30 case reference is made to fusion "from without", or an infected cell which expresses the envelope glycoproteins at its surface may fuse with an adjacent cell (fusion "from within").

35 The envelope determinants and the sequence of events causing the conformational changes in the envelope during the processes of fusion "from without" are well documented for orthomyxoviruses which require an acid

- 2 -

environment in the endocytosis vesicles in order to enter (Skehel, J.J. et al., PNAS, 79:968-972 (1982)). For retroviruses, for which the pathway of entry is independent of the pH, the precise determinants and steps leading from the recognition of the receptor to the activation of the fusion have not yet been elucidated. Other retroviruses are known to induce cell-to-cell fusion ("fusion from within"), such as the feline leukemia virus, the mouse mammary tumor virus, the avian reticuloendotheliosis virus, HIV and SIV.

Moreover, Fefferery S. Jones and Rex Risser (Journal of Virology, Jan. 1993, p. 67-74) have shown that the envelope glycoproteins of the wild-type ecotropic murine leukemia virus (MuLV), under the control of the viral LTR, are capable of inducing the formation of syncytia in rat XC cells in the absence of virions (fusion "from within").

To the inventors' knowledge, the envelope glycoproteins of a human endogenous retrovirus have never been shown to have fusogenic power in a process of fusion "from within".

Some authors have indeed put forward the hypothesis that the endogenous retroviral envelope of ERV3, a human endogenous retrovirus close to MLV (Moloney leukemia virus), may be involved, *in vivo*, in the development of the placenta via a process of fusion (Patrick J.W. Venables et al., Virology, 211, 589-592 (1995)), but this phenomenon has never been demonstrated *in vitro*. Furthermore, studies on the polymorphism of ERV3 env on individuals of Caucasian origin, have made it possible to demonstrate the presence of a mutation in the (SU) region of the ERV3 envelope, generating an early stop codon present in the homozygous state in 1% of the population studied, without these individuals exhibiting any abnormality of pregnancy or of placental development (Nathalie de

- 3 -

Parseval and Thierry Heidmann, Journal of Virology, Vol. 72, No. 4, pages 3442-3445 (1998)), this casting doubt over the hypothesis previously put forward.

- 5 The present inventors have now demonstrated, *in vitro*, that the unmodified HERV-W envelope glycoprotein, expressed under the control of a promoter, preferably a heterologous promoter, has fusogenic properties.
- 10 HERV-W is a recently described multicopy family of human endogenous retroviruses, so named because of the homology between the attachment site for the reverse transcription primer and that of avian retroviruses which use the Trp tRNA. No competent entity for its
- 15 replication has been demonstrated. The functionality of a promoter region has been verified and, among various healthy human tissues tested, its expression appears, by Northern blot, to be restricted to the placenta (J.L. Blond et al., Journal of Virology, Vol. 73,
- 20 No. 2, pages 1175-1185 (1999)). A single open reading frame coding for a potentially functional retroviral envelope exists on chromosome 7. A cDNA clone probably corresponding to a subgenomic transcript and bearing the complete sequence of the envelope has been isolated
- 25 from placental material (clone cl.PH74, GenBank AF072506, the sequence of which is identified by SEQ ID No. 2). The phylogenetic studies carried out at the protein level indicate that the envelope protein is type D. The sequence SEQ ID No. 2 given at the end of
- 30 the description therefore corresponds to the complete cDNA nucleotide sequence of the clone cl.PH74, the protein sequence of which is identified by SEQ ID No. 1.
- 35 Env HERV-W has all the "attributes" of a retroviral envelope: in particular, a leader peptide and the two characteristic subunits SU and TM separated by a furin cleavage site and, in its TM, it has a hydrophobic fusion peptide, an immunosuppressive region and a

- 4 -

transmembrane carboxyl region followed by a long cytoplasmic tail. Env HERV-W expression has been demonstrated in the placenta.

5 The experiments carried out by the inventors show that Env HERV-W causes, by cell-to-cell fusion, the formation of syncytia in various cell lines tested, of human and simian origin. The fusion phenomenon observed is dependent on the recognition of specific
10 receptor(s), as shown directly in transfections and indirectly in cocultures of transfected cells with other cell types. The present inventors have, moreover, identified the specific receptor for Env HERV-W using a competition approach based on the property of
15 interference of retroviral envelopes, by blocking cellular receptors with an envelope protein other than Env HERV-W, thus preventing the formation of syncytia. The receptor identified by the present inventors is the hATB^o receptor for type D mammalian retroviruses, which
20 is expressed in human cells (Rasko E.J. et al. PNAS, 1999, 96: 2129-2134 and Tailor C.S. et al. J. Virol., 1999, 73(5): 4470-4474). The use of this receptor, the method for the demonstration of which is described in one of the examples, is also part of the present
25 invention.

Thus, a subject of the present invention is a method for detecting the expression of an envelope protein or polypeptide of a human endogenous retrovirus, according
30 to which the protein or polypeptide has a polypeptide sequence which comprises the sequence SEQ ID No. 1 or a fragment of SEQ ID No. 1, or a sequence which exhibits, for any series of 20 amino acids, at least 80%, preferably at least 90%, or even at least 95% identity
35 with the sequence SEQ ID No. 1 or with a fragment of SEQ ID No. 1, and according to which the fusogenic power of said protein or of said fragment in cells of a cellular tissue or of a cell culture is detected by demonstrating the formation of syncytia.

- 5 -

Another subject of the invention is a method for detecting the expression of an envelope protein or polypeptide of a human endogenous retrovirus, according to which the protein or polypeptide has a polypeptide
5 sequence which exhibits, for any series of 20 amino acids, at least 80%, preferably at least 90%, or even at least 95% identity with the sequence SEQ ID No. 1, and according to which the fusogenic power of said protein in cells of a cellular tissue or of a cell
10 culture is detected by demonstrating the formation of syncytia.

According to the invention, said protein or said polypeptide has a polypeptide sequence which comprises
15 the sequence SEQ ID No. 1 or a fragment of SEQ ID No. 1, or a sequence which exhibits, for any series of 20 amino acids, at least 80%, preferably at least 90%, or even at least 95% identity with the sequence SEQ ID No. 1 or with a fragment of SEQ ID No. 1.

20 According to the present invention, it is clearly understood that said protein or said polypeptide, or said fragments thereof, if they do not exhibit complete identity with SEQ ID No. 1 or its fragments, should
25 have a fusogenic power preferably at least equal to or greater than that of SEQ ID No. 1 or its fragments.

If the fragments of the protein or of the polypeptide of the invention exhibit complete identity with the
30 fragments of SEQ ID No. 1, then the size of these fragments may be less than 20 amino acids, for example it may be approximately 10 amino acids, or even approximately 5 amino acids.

35 The variations envisaged according to the invention in the polypeptide sequence of the protein or of the polypeptide, or of their fragments, comprise the variations linked to the polymorphism, but also modifications such as substitution(s), deletion(s) and

- 6 -

addition(s) which may be introduced into said polypeptide sequence in order to obtain a protein, a polypeptide, or a fragment thereof, which has fusogenic power, in particular at least equal to or greater than
5 that of SEQ ID No. 1 or its fragments.

The polymorphism analysis may be carried out by the SSCP (single strand conformational polymorphism) method, which is an electrophoretic method which makes
10 it possible to objectify, using differences in migration, the presence of at least one mutation which distinguishes two short sequences (less than 250 bp). Thus, as illustrated in figure 4, after amplification on total DNA using the primers U6198 and L6186, or
15 U6189 and L6186, it is possible to analyze the polymorphism of the envelope located on chromosome 7 using the set of primers represented (U6302 to L6321), making it possible to generate a set of 10 overlapping fragments of suitable size. The polymorphism of one of
20 the subfragments may also be demonstrated by sequencing, mapping and/or restriction techniques, as appropriate, or more simply by a sandwich hybridization technique of the ELOSA type which makes it possible to distinguish as little as a point mutation (Cros P. et
25 al., European patent application EP 0 486 661).

Examples of polymorphic Env HERV-W sequences are represented in the attached figure 1, the corresponding DNA sequences being represented in figure 2. These
30 figures represent the alignment of protein and nucleic acid sequences obtained by sequencing clones derived from three different individuals.

Moreover, the polymorphism of the LTR which directs the
35 transcription of the env gene located on chromosome 7 was studied. Two groups of 5' LTRs are observed, the nucleic acid sequences of which, obtained by sequencing two clones originating from two different individuals, are represented and aligned in figure 3.

- 7 -

A judicious choice of primers has made it possible to specifically amplify, on chromosome 7, from total human DNA, a nucleic acid fragment containing all the information U3RU5-gag-pol-env-U3RU5, using the U6198 and L6186 primers, or exclusively the env-U3RU5 sequence, using the U6189 and L6186 primers. Such an approach is, for example, possible using a primer which overlaps the zone where the retrovirus sequence (U3 upstream, U5 downstream) joins the contiguous nonretroviral flanking sequence. For example, the L6186 primer overlaps the terminal 3' U5 region and the downstream nonretroviral sequence. Using such a PCR product isolating the sequence of interest from the mixture of HERV-W sequences present in the human genome, it is possible to carry out an analysis of the polymorphism.

Preferentially, said protein has at least one of the following characteristics:

- it is encoded by the env gene of the HERV-W endogenous retrovirus;
- it is encoded by an open reading frame located on chromosome 7 of the human genome;
- it has a polypeptide sequence which comprises the sequence SEQ ID No. 1, or a sequence which exhibits, for any series of 20 amino acids, at least 80%, preferably at least 90%, or even at least 95% identity with SEQ ID No. 1. Preferably, it consists of SEQ ID No. 1.

30

Preferentially, the polypeptide has a polypeptide sequence which begins at amino acid 448 and ends at amino acid 538 of SEQ ID No. 1 or a polypeptide sequence which exhibits, for any series of 20 amino acids, at least 80%, preferably at least 90%, or even at least 95% identity with the polypeptide sequence which begins at amino acid 448 and ends at amino acid 538 of SEQ ID No. 1. Preferably, it consists of a polypeptide sequence which begins at amino acid 448 and

- 8 -

ends at amino acid 538 of SEQ ID No. 1. The polypeptide which corresponds to the above definition is a regulatory element which can confer or restore its fusogenic capacity on or to a retroviral envelope not
5 reputed to be fusogenic in a cell-cell fusion test.

The cells of said tissue or of said cell culture, in which demonstration of the fusogenic power is sought, are advantageously chosen from bone cells, muscle
10 cells, placenta cells, endothelial cells, in particular of blood vessels, epithelial cells, glial cells and tumor cells or cells derived from tumor cell lines.

As will be illustrated in the following examples, the
15 detection of the fusogenic power of said protein or of said polypeptide may be carried out according to at least any one of the following two protocols.

According to a first protocol, a vector for expression
20 of said protein or of said polypeptide is obtained, based on which the expression of the protein, of the polypeptide or of its gene is under the control of a promoter, preferably a strong promoter; cells are transfected with the vector obtained, so as to obtain
25 producer cells expressing, at their surface, said protein or said polypeptide; and the formation of syncytia or the absence of formation of syncytia is observed.

30 According to a second protocol, a vector for expression of said protein or said polypeptide is obtained, based on which the expression of the protein, of the polypeptide or of its gene is under the control of a promoter, preferably a strong promoter; cells are
35 transfected with the vector obtained, so as to obtain producer cells expressing, at their surface, said protein or said polypeptide; naïve indicator cells expressing, at their surface, a receptor for said protein are cocultured in the presence of said producer

- 9 -

cells; and the formation of syncytia or the absence of formation of syncytia is observed.

5 The present invention also relates to the use of a gene or of a nucleic acid, or of a fragment of gene or of a nucleic acid, coding for a protein or a polypeptide as defined above in the description of the methods which are subjects of the invention, under suitable conditions which allow its expression, for preparing a
10 therapeutic or prophylactic composition.

Another subject of the invention is a therapeutic or prophylactic composition comprising a gene or a nucleic acid, or a fragment of gene or of nucleic acid, coding
15 for a protein or a polypeptide as defined above.

Such a composition may also comprise a heterologous or autologous promoter, preferably a heterologous promoter, for the expression of said protein or of said
20 polypeptide.

The invention also relates to the following subjects:

- an expression vector comprising at least one gene or one nucleic acid, or one fragment of gene or of
25 nucleic acid, coding for a protein or a polypeptide as defined above, and elements required for its expression in a host cell;
- a host cell comprising at least one expression vector of the invention, and
- 30 - a therapeutic or prophylactic composition comprising at least one expression vector or one host cell of the invention.

The various therapeutic compositions of the invention
35 are in particular intended for the treatment of cancers, such as by destroying the cancer cells by means of the formation of syncytia. The various prophylactic compositions of the invention are in

- 10 -

particular intended to prevent a deficiency in placental development.

5 The therapeutic or prophylactic compositions of the invention, as defined above, are advantageously intended for a treatment commonly named "treatment by gene therapy" or "treatment by gene transfer".

10 As stated above, the fusogenic properties of the Env HERV-W protein, of the Env HERV-W polypeptide or of their fragments as defined in the present invention in particular find an application in the domain of cancer gene therapy.

15 To date, the genes most commonly used in therapy against cancers are (i) the genes which code for proteins which increase the immunogenicity of the tumor cells, such as pro-inflammatory cytokines, (ii) the genes which code for enzymes which make the cancer
20 cells sensitive to a promedicament in gene/prodrug systems, such as the Herpes Simplex virus thymidine kinase/Ganciclovir system or the cytosine deaminase/5FC system.

25 Ideally, the transfer of therapeutic genes should lead both to a local destruction of the cancer cells and to activation of antitumor immunity in order to eliminate the tumor regions to which the therapeutic genes cannot be delivered, and the treatment should not cause damage
30 to the host's normal cellular tissues, in particular to the tissues of the vital organs.

The protein or polypeptide of the invention, which comprises or consists of the Env HERV-W protein or its
35 fragments, in particular a fragment which begins at amino acid 448 and ends at amino acid 538 of SEQ ID No. 1, or of a polypeptide sequence which has, for any series of 20 amino acids, at least 80%, preferably at least 90%, or even at least 95% identity with SEQ ID

- 11 -

No. 1 or a fragment of SEQ ID No. 1, and in particular the fragment identified above, under the control of a heterologous or autologous promoter capable of inducing its expression, corresponds to the criteria defined
5 above by the formation of syncytia. The syncytia form from one or more transfected cell(s) by a process of cell-to-cell fusion.

In an embodiment with a view to optimizing its
10 therapeutic characteristics, the protein or polypeptide of the invention, or any fragment, is optionally fused with one or more other protein(s) or protein fragment(s), even if intrinsically it corresponds to the criteria defined above. On the other hand, all or
15 part of the protein, and in particular the polypeptide the peptide sequence of which comprises or consists of the sequence which begins at amino acid 448 and ends at amino acid 538 of SEQ ID No. 1, may be fused with other proteins with a view to conferring on them particular
20 properties. The protein or polypeptide of the invention, or its fragment, is capable of inducing the formation of syncytia at a pH close to neutral or at neutral pH. Typically, the expression vector or plasmid will be adjusted to allow expression of the protein,
25 the polypeptide or the fragment which induces the formation of syncytia, such that, when it is expressed, the protein, polypeptide or fragment may induce the fusion of transfected cells with other nontransfected human cells. It is desirable for the protein or
30 polypeptide of the invention to be expressed independently of other viral components, unless these components are useful for the vectorization.

Thus, a subject of the present invention is a gene or a
35 nucleic acid, or a fragment of gene or of a nucleic acid, which is recombinant and which codes for a protein, a polypeptide or a fragment of the invention which induces the formation of syncytia by fusion of transformed cells and target malignant cells, and its

- 12 -

use in the domain of therapy for malignant diseases, such as cancers.

5 The invention also relates to a method for treating a malignant disease in a patient, which consists in administering to the patient the gene or a nucleic acid, or a fragment of gene or of a nucleic acid, which is recombinant and which codes for a protein, a polypeptide or a fragment of the invention which
10 induces the formation of syncytia by fusion of transformed cells and target malignant cells.

The gene or the nucleic acid, or the fragment of gene or of nucleic acid, is introduced *in vitro* into
15 suitable human cells, such as cells of immortalized continuous lines, by standard techniques known to those skilled in the art, such as transfection, transduction or transformation, and the cells thus transformed are then introduced into the patient, where they may exert
20 their fusogenic properties.

The gene or the nucleic acid, or the fragment of gene or of nucleic acid, of the invention may be used in various ways for the treatment of cancers, in
25 particular for the treatment of solid or soft tumors. The target cells may be transformed *ex vivo* or *in vivo* with the vectors (plasmids) coding for the polypeptide of the invention.

30 The fusogenic properties of the Env HERV-W protein, of the Env HERV-W polypeptide or of their fragments as defined in the present invention also find an application in the domain of prophylaxis, for preventing a deficiency in placental development and
35 overcoming failed pregnancies.

The gene or the nucleic acid or their fragments as defined in the invention may therefore be used for various therapeutic or prophylactic effects, the

- 13 -

ultimate aim being (i) to destroy the target cells by formation of syncytia inducing cell death in the target cells by a process of death other than cell death by apoptosis, or (ii) to induce or to promote the formation of syncytia, for example to overcome a deficiency in the formation of syncytiotrophoblasts during pregnancy, or to prevent a deficiency in the natural formation of any other type of syncytia, said deficiency being associated with a pathology.

10

The invention also relates to the use of the Env HERV-W protein, or of a fragment of Env HERV-W, as defined above, at the surface of a gene therapy vector comprising, inter alia, a gene, a nucleic acid sequence or an oligonucleotide of therapeutic interest capable of being expressed in a target cell or of hybridizing to a complementary nucleotide sequence from a target cell, said Env HERV-W protein or said fragment of this protein interacting with its cellular receptor described above, thus promoting the introduction of the gene, the nucleic acid sequence or the oligonucleotide of therapeutic interest into the target cell.

20

Thus, the invention relates to a gene therapy vector comprising an envelope protein, polypeptide or fragment of a human endogenous retrovirus, said protein or said polypeptide having a polypeptide sequence which comprises the sequence SEQ ID No. 1 or a fragment of SEQ ID No. 1, in particular a fragment the peptide sequence of which comprises or consists of the sequence which begins at amino acid 448 and ends at amino acid 538 of SEQ ID No. 1, or a sequence which exhibits, for any series of 20 amino acids, at least 80%, preferably at least 90%, or even at least 95% identity with the sequence SEQ ID No. 1 or with a fragment of SEQ ID No. 1, in particular as defined above. Preferably, the gene therapy vector of the invention comprises the sequence SEQ ID No. 1. In a particular embodiment of the invention, the gene therapy vector mentioned above

25

30

35

- 14 -

consists of a conventional retroviral vector of the MLV type or of a lentiviral vector pseudotype with all or part of the envelope protein of HERV-W as defined above, or alternatively of a synthetic vector carrying,
5 at its surface, all or part of the Env HERV-W protein as defined above which confers the properties of cell targeting and of plasma membrane fusion.

The invention also relates to a gene therapy vector
10 comprising, at its surface, the receptor for the protein identified in SEQ ID No. 1, in particular for targeting cells producing the protein identified in SEQ ID No. 1 in a constitutive or induced manner.

15 The nucleic acid sequences and/or oligonucleotides of therapeutic interest (antisense or coding for a protein) in particular make it possible to target the cells in which a gene is expressed.

20 The antisense nucleic acid sequences or oligonucleotides are capable of interfering specifically with the synthesis of a target protein, by inhibiting the formation and/or the functioning of the polysome, depending on the position of the antisense in
25 the mRNA of the target. Therefore, the common choice of the sequence surrounding the translation initiation codon as a target for inhibition by an antisense nucleic acid sequence or by an antisense oligonucleotide is aimed at preventing the formation of
30 the initiation complex. Other mechanisms in the inhibition by antisense oligonucleotides involve activation of ribonuclease H, which digests the antisense oligonucleotide/mRNA hybrids, or interference at splicing sites by antisense oligonucleotides whose
35 target is an mRNA splicing site. The antisense oligonucleotides are also complementary to DNA sequences and may therefore interfere at the level of transcription, by forming a triple helix, the antisense oligonucleotide pairing via "Hoogsteen" hydrogen bonds

- 15 -

at the level of the major groove of the DNA double helix. In this particular case, reference is made more precisely to anti-gene oligonucleotides. It is clearly understood that the antisense nucleic acid sequences or
5 oligonucleotides may be strictly complementary to the DNA or RNA target to which they must hybridize, but also not strictly complementary, on the condition that they hybridize on the target. Similarly, they may be antisense oligonucleotides which may or may not be
10 modified at the level of the internucleotide bonds. All these notions are part of the general knowledge of those skilled in the art.

The present invention therefore relates to a
15 therapeutic composition comprising, inter alia, a gene therapy vector, the Env HERV-W protein or a fragment of this protein as defined above, and an antisense nucleic acid sequence or oligonucleotide as defined above.

20 The Env HERV-W protein or one of its fragments is also used as a therapeutic vector for the transfer of a gene of therapeutic interest into a target cell and in the formulation of a therapeutic composition comprising at least one gene therapy vector, the Env HERV-W protein
25 or a fragment of this protein as defined above, and a gene of therapeutic interest, and also the elements which allow the expression of said gene of therapeutic interest. The genes of therapeutic interest may be nonmutated or mutated. They may also consist of nucleic
30 acids modified such that it is impossible for them to integrate into the genome of the target cell, or nucleic acids stabilized with agents, such as spermine.

The expression "elements which ensure the expression of
35 said gene of therapeutic interest *in vivo*" refers in particular to the elements required to ensure the expression of said therapeutic gene after it has been transferred into a target cell. They are, in particular, promoter sequences and/or regulatory

- 16 -

sequences which are effective in said cell and, optionally, the sequences required to allow the expression of a polypeptide at the surface of the target cells. The promoter used may be a viral,
5 ubiquitous or tissue-specific promoter or a synthetic promoter.

By way of example, mention will be made of promoters such as the RSV (Rous Sarcoma Virus), MPSV, SV40
10 (Simian Virus), CMV (Cytomegalovirus) or vaccinia virus promoters. It is also possible to choose a promoter sequence specific for a given cell type or activatable under defined conditions. The literature provides a great deal of information relating to such promoter
15 sequences.

In another embodiment, use may be made, in a therapeutic composition, of a cell expressing the Env
HERV-W protein or a fragment of this protein as defined
20 above, as a vehicle for one or more gene(s) which is large in size, due to the fusogenic properties of the protein or of its fragments, which allow the vector cell to fuse with a host cell deficient for one or more given genes, thus making it possible to compensate for
25 the deficient gene(s) (example: dystrophin).

The invention therefore also relates to such a cell and its use as a cellular vector.

30 The fusogenic properties or power of the protein or of the polypeptide of the invention, or of their fragments, are also used in a method for testing the effectiveness of and selecting medicinal substances or drugs, or gene/prodrug systems, capable of having a
35 qualitative and/or quantitative effect on their fusogenic power, by bringing said medicinal substance or drug, or said gene/prodrug system, into contact with cells of a cell culture expressing said protein or said polypeptide or said fragment, and observing a

- 17 -

5 regression in or a disappearance of the formation of syncytia, it being understood that the formation of syncytia in the natural state is associated with a pathological condition. By way of example, mention may be made of hemorrhagic phenomena, the destruction or modification of neuronal cells, and the exacerbated modification or destruction of osteoblasts.

10 The invention also relates to a method for selecting medicinal substances or drugs, or gene/prodrug systems, capable of having a qualitative and/or quantitative effect on the fusogenic power of a protein or of a polypeptide or of a fragment as defined above. According to this method, said medicinal substance or
15 drug, or said gene/prodrug system, is brought into contact with cells of a cell culture expressing said protein or said polypeptide or said fragment, and a regression in or a disappearance of the formation of syncytia is observed.

20 The invention also relates to the use of at least one antisense nucleic acid sequence or of at least one antisense oligonucleotide corresponding to the criteria described above and capable of hybridizing and of
25 interfering specifically with the synthesis of the Env HERV-W protein, and to a therapeutic composition comprising, inter alia, said antisense nucleic acid sequence or oligonucleotide, with the aim of obtaining, *in vivo*, a regression or a disappearance of syncytia
30 associated with a pathological condition.

With the aim of obtaining, *in vivo*, regression of the formation of syncytia or disappearance of syncytia associated with a pathological condition, a therapeutic
35 composition is prepared which comprises, inter alia, a ligand capable of recognizing the receptor identified above and of inactivating or inhibiting the process of formation of syncytia, or a composition is prepared which comprises a gene coding for a ligand capable of

- 18 -

being expressed, *in vivo*, in a target cell or in a given target cell tissue, said gene being under the control of the required elements which ensure its expression after it has been transferred into the
5 target cell or cellular tissue.

Thus, the term "ligand" is intended to mean any molecule which is capable of recognizing said receptor and/or of inhibiting its function. It may be, *inter*
10 alia, a monoclonal antibody or a polyclonal antibody, or a monoclonal antibody or polyclonal antibody fragment. It may also be a molecule which inhibits the function of the receptor, the affinity constant of which would be greater than that of the Env HERV-W
15 protein for its binding and attachment to the receptor.

The production of polyclonal and monoclonal antibodies is part of the general knowledge of those skilled in the art. Mention may be made, by way of reference, of
20 Köhler G. and Milstein C. (1975): Continuous culture of fused cells secreting antibody of predefined specificity, *Nature* 256: 495-497 and Galfre G. et al. (1977) *Nature*, 266: 522-550, for the production of monoclonal antibodies, and Roda A., Bolelli G.F.:
25 Production of high-titer antibody to bile acids, *Journal of Steroid Biochemistry*, Vol. 13, pp 449-454 (1980), for the production of polyclonal antibodies. For the production of monoclonal antibodies, an immunogen may be coupled to Keyhole Limpet Hemocyanin
30 (KLH peptide) as a support for the immunization, or to serum albumin (SA peptide). The animals are given an injection of immunogen using complete Freund's adjuvant. The sera and the hybridoma culture supernatants derived from the immunized animals are
35 analyzed for their specificity and their selectivity, using conventional techniques, such as for example ELISA or Western blot assays. The hybridomas producing the most specific and the most sensitive antibodies are selected. Monoclonal antibodies may also be produced

- 19 -

in vitro by cell culture of the hybridomas produced or by recovery of ascites fluid, after intraperitoneal injection of the hybridomas into mice. Whatever the method of production, by supernatant or by ascites, the antibodies are then purified. The purification methods used are essentially ion-exchange gel filtration and exclusion chromatography or immunoprecipitation. A number of antibodies sufficient to identify the most effective ones are screened in functional assays. The *in vitro* production of antibodies, of antibody fragments or of antibody derivatives, such as chimeric antibodies produced by genetic engineering, is well known to those skilled in the art.

More particularly, the term "antibody fragment" is intended to mean the F(ab)₂, Fab, Fab' or sFv fragments (Blazar et al., 1997, Journal of Immunology 159: 5821-5833 and Bird et al., 1988, Science 242: 423-426) of a native antibody, and the term "derivative" is intended, *inter alia*, to mean a chimeric derivative of a native antibody (see for example Arakawa et al., 1996, J. Biochem 120: 657-662 and Chaudray et al., 1989, Nature 339: 394-397).

As mentioned above, gene therapy opens up the possibility of expressing such ligands *in vivo*, by administering therapeutic compositions comprising at least one gene coding for such a ligand. Such a gene of therapeutic interest codes, in particular, (i) either for at least one polyclonal or monoclonal antibody, or a monoclonal or polyclonal antibody fragment, or for a native transmembrane antibody, or a fragment of such an antibody, provided that the antibody or antibody fragment is expressed *in vivo* at the surface of a target cell or of target cells of a tissue and is capable of recognizing and of binding to said receptor, (ii) or for at least one inhibitory molecule as described above.

- 20 -

The expression "target cells" or "target cells of a tissue", as defined above, is intended to mean (i) either cells at the level of which the intention is to act so as to prevent or inhibit the formation of syncytia, (ii) or cells other than these but which are capable of expressing the ligand and, consequently, of inhibiting and/or blocking the functional activity of the receptor.

10 The expression "element which ensures the expression *in vivo* of said gene" refers in particular to the elements required to ensure its expression after it has been transferred into a target cell. They are, in particular, the promoter sequences and/or the
15 regulatory sequences which are effective in said cell and, optionally, the sequences required to allow the expression, at their surface [sic], of an inhibitory polypeptide or molecule, as mentioned above. The promoter used may be a viral, ubiquitous or tissue-specific promoter or a synthetic promoter. Examples of
20 such promoters have been described previously.

The term "transmembrane antibody" is intended to mean an antibody in which at least the functional region
25 capable of recognizing and of attaching to the receptor is expressed at the surface of the target cells so as to allow recognition and attachment. Such antibodies may consist of fusion polypeptides comprising an amino acid sequence defining the functional region and an
30 amino acid sequence defining a transmembrane polypeptide which allows the anchoring within the lipid bilayer of the membrane of the target cells or to the external surface of this lipid bilayer. Nucleic acid sequences coding for such transmembrane antibodies are
35 described in the literature.

The expression "gene or nucleic acid sequence or their fragments" is intended to mean (i) an isolated native gene or nucleic acid or their isolated fragments

- 21 -

obtained by enzymatic cleavage, or (ii) a gene or nucleic acid or their fragments obtained by chemical synthesis using automatic synthesizers, such as the synthesizers marketed by Applied Biosystems.

5

The term "tumor cells" is intended to mean (i) cells of immortalized cell lines or (ii) primary tumor cells removed from a patient.

10

The term "autologous promoter" is intended to mean a 5' LTR of HERV-W, on the condition that it is functional, and the term "heterologous promoter" is intended to mean any promoter which does not belong to the HERV-W family, of viral, retroviral or cellular origin, optionally modified, on the condition that it is functional. Advantageously, the autologous or heterologous promoter is a strong promoter, i.e. it is capable of inducing quantitatively significant expression of the protein or of the polypeptide.

20

The fusogenic power of the Env HERV-W protein may also be used to promote the process of cell adhesion in the case of heterologous or homologous grafts or in cell repair processes.

25

Example 1:

Cell lines:

The TELCeB6 line (Cosset et al., Journal of Virology, 69 (12): 7430-7436 (1995)) derives from the TELac2 line after transfection and clonal selection of an expression plasmid intended to produce Gag and Pol protein of the MoMLV (Moloney murine leukemia virus) type. The TELac2 line initially derives from human rhabdomyosarcoma cells TE671 (ATCC CRL 8805) and expresses the nlsLacZ retroviral reporter vector (Takeuchi et al., Journal of Virology, 68 (12): 8001-8007 (1994)). The production of infectious retroviral

30

35

- 22 -

particles by TELCeB6 cells depends on the envelope expression vectors transfected.

5 The cells are cultured in DMEM medium (Dulbecco modified Eagle medium - Life Technologies) with 10% of fetal calf serum (Life Technologies). In general, this medium was used for all the other cell types, i.e. the TE671 (ATCC CRL 8805 - human rhabdomyosarcoma), A-431 (ATCC CRL-1555 - solid tumor, human epidermoid carcinoma), HeLa (ATCC CCL-2), COS (ATCC CRL-1651), PAE (pig aorta endothelial cells), XC (ATCC CCL-165 - rat sarcoma), NIH-3T3 and QTB (ATCC CRL-1708) cells.

Construction of the envelope expression vectors:

15 The pHCMV plasmid was used for the expression of env HERV-W. The FBASALF-ARless plasmid was used as a positive control for fusion; it produces a highly fusogenic form of the amphotropic MLV envelope glycoprotein, modified by introducing a stop codon before the first amino acid of the intracytoplasmic peptide p2-R (Rein et al., Journal of Virology, 68 (3): 1773-1781 (1994)). env HERV-W cloned, in the antisense direction, into the pHCMV plasmid was used as a negative control.

25

Transfection and cell-to-cell fusion tests (coculture): The envelope glycoprotein expression plasmids are transfected into the TELCeB6 cells by calcium phosphate precipitation (Cosset et al., Journal of Virology, 69 (10): 6314-6322 (1995)). The confluent TELCeB6 cells expressing Env are fixed with 0.5% glutaraldehyde in PBS, 24 h after transfection. Staining with May-Grünwald and Giemsa solutions (MERCK) is then carried out according to the supplier's recommendations. It stains the nuclei violet and the cytoplasms mauve and enables the syncytia to be visualized.

35

For the coculture experiments, the transfected cells are detached from the support, counted and then re-

- 23 -

seeded at equal concentration (3×10^5 cell/well) in 6-well plates. Fresh indicator cells are then added to the transfected cells, at 10^6 per well, and the coculture is allowed to continue for 24 h. XGal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside) staining may then be carried out in order to stain the nucleus of the TELCeB6 cells (Cosset et al., Journal of Virology, 69 (10): 6314-6322 (1995)). It is followed by staining with May-Grünwald and Giemsa solutions (MERCK), carried out according to the supplier's recommendations.

Most of the syncytia can be observed 18 to 24 hours after the beginning of transfection; the progressive detachment of the cells no longer allows observation or staining 36 hours after transfection. The fusion observed corresponds to a fusion "from within", i.e. a cell-to-cell fusion, based on a cell expressing the envelope, as opposed to a fusion "from without" which corresponds to formation of syncytia subsequent to a virion-cell(s) fusion.

Table I below gives the results obtained regarding the capacity for cell-to-cell fusion of Env HERV-W by direct transfection, compared to that of the ARless control envelope. The TELCeB6 and TE671 cells correspond to lines of human origin. The COS cells are green monkey kidney cells. The XC cells are rat cells.

Table I

Envelope	TELCeB6 cells	fusion index ^a on		
		TE671 cells	COS cells	XC cells
Arless	33	8.6	inn.	40
HERV-W	61	24.7	36	0

^a The fusion index corresponds to the percentage $(N-S)/T$, in which N is the number of nuclei in

- 24 -

syncytia, S is the number of syncytia and T is the total number of nuclei counted. inn. signifies innumerable, organized in a "network".

- 5 Table I shows that the results for Env HERV-W are at least as good as for the control on the cells of human origin. They are less good on the simian cells. Env HERV-W does not induce the formation of syncytia on rat cells.

10

Table II below gives the data observed in experiments of coculturing indicator cells with TELCeB6 cells transfected with pHCMV-env HERV-W. The type, origin and species of the indicator cells are indicated. The

15 formation of syncytia is indicated by the term yes/no.

Table II

Species	Cell type	Origin	Fusion in coculture with TELCeB6
Human	TE671	Rhabdomyosarcoma	Yes
	A431	Epidermoid carcinoma	Yes
	HeLa	Epithelioid carcinoma	Yes
Monkey	COS	Fibroblast type	Yes
Pig	PAE	Endothelium	Yes
Rat	XC	Sarcoma	No
Mouse	3T3	Fibroblastic	No
Quail	QT6	Fibrosarcoma	No

- 20 Table II gives the results of the coculture experiments as a function of the cell lines tested. Syncytia are observed in human rhabdomyosarcoma (TE671), epidermoid carcinoma (A431) and epithelioid carcinoma (HeLa) cells, and also in monkey cells of the fibroblast type
- 25 (COS), pig endothelium cells (PAE) and mouse cells of the fibroblast type (3T3). The fact that the human endogenous envelope Env HERV-W is capable of fusing in pig cells may pose problems in the context of organ transplantation (xenotransplantation).

- 25 -

Example 2:

Joint and then selective amplification of the LTR and of the envelope:

In order to study the polymorphism of the coding region of the envelope and of the associated 5' LTR U3 promoter region, located on chromosome 7, amplification specific for a 10 kb fragment is carried out using a pair of specific primers. In fact, given that the HERV-W family comprises many noncoding copies and in particular a considerable number of LTRs, this strategy makes it possible to specifically and jointly amplify the env region and its promoter sequence (5' LTR) located upstream, exclusively on chromosome 7. For this, use is made of a primer U6198 which hybridizes on a specific sequence located upstream of 5' LTR on chromosome 7, and a primer L6186 which hybridizes in an overlapping manner on the 3' LTR U5 region and the adjacent cellular gene, on this same chromosome. Long distance PCR (or LD-PCR) is carried out under the following conditions, 1 x 5 min at 94°C, 10 x (10 sec at 94°C, 30 sec at 55°C, 8 min at 68°C), 25 x (10 sec at 94°C, 30 sec at 55°C, 8 min at 68°C + 10 sec/cycle), 1 x 7 min at 68°C, in the presence of amplification buffer (50 mM Tris HCL, pH 9.0, at 25°C, 15 mM (NH₄)₂SO₄, 0.1% Triton X-100); 1.5 mM MgCl₂, 0.25 mM of each dNTP, 330 nM of each primer (U6198 and L6186), 1U of DNA polymerase and also 200 ng of matrix (genomic DNA) in a final volume of 50 ml.

A nested "env" PCR and also a nested "LTR" PCR are carried out using this diluted 10 kb PCR product, in order to objectify the presence or absence of a polymorphism of these two regions. The dilution allows specific amplification from the LD-PCR product and not from the starting genomic material. The nested "env" PCR is carried out using the U6189 and L6186 primers, the U6189 [sic] primer being that used for the LD-PCR, the U6189 primer being located upstream of the env ATG. The 5' LTR U3 region is amplified with the U6460 and

- 26 -

L5643 pair of primers. The U6460 primer hybridizes upstream of the 5' LTR, while the L5643 primer hybridizes in the R domain of the 5' LTR. The nested PCRs are carried out under the following conditions,
5 1 × 5 min at 94°C, 30 × (1 min at 94°C, 1 min at 53°C, 3 min at 72°C), 1 × 7 min at 72°C, in the presence of amplification buffer (10 mM Tris HCL, pH 8.3, 50 mM KCl), 1.5 mM MgCl₂, 0.25 mM of each dNTP, 330 nM of each primer, 1.25U of DNA polymerase and an aliquot of
10 the LD-PCR amplification product, in a final volume of 50 ml.

Analysis of the polymorphism:

In order to objectify the presence or absence of a
15 polymorphism, the nested PCR products can be analyzed in various ways, in particular sequencing or analysis by the SSCP (Single Strand Conformation Polymorphism) technique which makes it possible to demonstrate the presence of at least one mutation between two short
20 sequences with a mean size of 250 bp.

Polymorphism of the env gene: the use of 20 primers (10 even sense primers: 6302 to 6320, and 10 odd antisense primers: 6303 to 6321) makes it possible to sequence
25 the coding region of the envelope using the nested envelope PCR product. These primers may also be used for analysis of the polymorphism by SSCP. By way of example, the sequences of the envelope genes of three healthy donors labeled D6, D10 and D21, are illustrated
30 in figure 2. These sequences show the existence of a low polymorphism rate. If the envelope sequence of donor D6 is used as an arbitrary reference, the sequence of the envelope of donor D21 has a mutation at position 386 (T386C), the replacement of the thymine
35 with cytosine inducing an amino acid change of valine to alanine (V128A by protein numbering). Similarly, the sequence of the envelope gene of donor D10 has two mutations relative to the sequence of donor D6, at position 671 (T671C) and 920 (G920A), inducing two

- 27 -

amino acid changes, from valine to alanine (V224A by protein numbering) and from serine to asparagine (S306N by protein numbering), respectively. These sequences illustrate the existence of a polymorphism. 12 patient
5 DNAs were sequenced, which made it possible to observe a low polymorphism rate between the DNAs tested. For example, comparison of the sequences derived from two individuals, noted 10 and 21, shows the presence of a nucleic acid difference of three bases over the 1617
10 bases of the gene, which corresponds to a polymorphism rate of 0.19%. Two mutations are located on the sequence of DNA 10 (T671C and G920A) and one on the sequence of DNA 21 (T386C). The sequence of individual 6 is used as the reference. This same analysis at the
15 protein level makes it possible to observe 3 mutated amino acids for the entire envelope comprising, in total, 538 amino acids, i.e. a polymorphism rate of 0.56%. The two mutations of the sequence derived from individual 10 are V224A and S306N, and that of the
20 sequence derived from individual 21 is V128A.

Polymorphism of the LTR5' U3 promoter region associated with the envelope gene: the sequencing of the 5' LTR U3 domain is carried out using the 2 primers previously
25 used for the nested LTR PCR. By way of example, the sequences of the 5' LTR U3 region (associated with the envelope gene) of two of the healthy donors (labeled D6 and D21), for which the envelope has, moreover, been sequenced, are illustrated in figure 3. These sequences
30 show the existence of a polymorphism rate which is higher than for the envelope gene. The variations at positions 210 (T for D6, C for D21), 211 (G for D6, A for D21), 229 (A for D6, G for D21), 231 (T for D6, C for D21) and 232 (C for D6, A for D21) will in
35 particular be noted.

The sequences of the primers used for the PCR, the SSCP and the sequencing are illustrated in table III below.

- 28 -

Table III

NAME :	NUCLEOTIDE SEQUENCES :
Long distance PCR primers	
U6198 :	5'- CAA-AAC-GCC-TGG-AGA-TAC-AGC-AAT-TAT-C-3'
L6186 :	5'- GCA-CCC-TCA-TGG-TTG-TGT-TAC-TTG-G-3'
Nested <i>env</i> PCR primers	
U6189 :	5'- CTG-AAA-ATC-CAG-GAG-ACA-ACG-CTA-GC-3'
L6186 :	5'- GCA-CCC-TCA-TGG-TTG-TGT-TAC-TTG-G-3'
Nested 5' LTR PCR primers	
U6460 :	5'- TTG-GTA-CCC-AAA-ACG-CCT-GGA-GAT-ACA-GCA-ATT-ATC-3'
L5643 :	5'- AAC-TCG-AGT-GAA-ATA-GCA-TGA-AAA-CAG-AG-3'
SSCP and <i>env</i> sequencing primers	
U6302 :	5'- AGG-AAA-GTA-ACT-AAA-ATC-ATA-AAT-C-3'
L6303 :	5'- GGT-TCC-CTT-AGA-AAG-ACT-CC-3'
U6304 :	5'- AAT-ATT-GAT-GCC-CCA-TCG-TAT-A-3'
L6305 :	5'- CCA-GTT-TGG-GTG-AAG-TAA-GTC-3'

- 29 -

U6306 :	5'- GGA-GGA-CTT-GGA-GTC-ACT-GTC-3'
L6307 :	5'- AGG-CGA-GTA-TGG-GTA-CGG-AG-3'
U6308 :	5'- GGA-CTA-GAT-CTC-TCA-AAA-CTA-CA-3'
L6309 :	5'- ACG-GAA-GTG-GTG-TTT-ATT-TCT-G-3'
U6310 :	5'- CCT-GAA-CAA-TGG-AAC-AAC-TTC-3'
L6311 :	5'- ATT-CCT-GAG-GGT-AGG-CAG-AC-3'
U6312 :	5'- GGT-AAC-TCC-TCC-CAC-ACA-AA-3'
L6313 :	5'- GAA-TGG-GTA-CTC-TTT-TGT-TGC-3'
U6314 :	5'- TAC-AGT-TAT-GTC-ATA-TCT-AAG-CC-3'
L6315 :	5'- TAA-GTT-GAT-CTT-GCA-AGG-TGA-C-3'
U6316 :	5'- CTA-AAT-GGG-GAC-ATG-GAA-CG-3'
L6317 :	5'- TAT-TCG-ATC-TGG-AAT-TTC-TTC-AAC-3'
U6318 :	5'- CAA-TCC-GGA-ATC-GTC-ACT-GA-3'
L6319 :	5'- AGA-CAA-AGT-TAA-CAA-GGA-GGT-TC-3'
U6320 :	5'- ACT-CCT-CTT-TGG-ACC-CTG-TAT-C-3'
L6321 :	5'- GAG-GTT-GGC-CGA-CCA-CCG-3'

U refers to sense primers and L refers to reverse primers.

5

Example 3:

Interference tests were carried out in order to determine the receptor recognized by the envelope glycoprotein of HERV-W among the receptors known to be expressed in human cells, i.e. PiT-2 (the receptor for amphotropic MLVs), PiT-1 (the receptor for GALV - gibbon ape leukemia virus and FeLV-B - feline leukemia virus type B) and hATB^o (the receptor for type D mammalian retroviruses, also recognized by the RD114 retrovirus). For this, TELCeB6 cells were transfected either with the expression plasmid coding for the HERV-W envelope, with the expression plasmid expressing the antisense messenger RNA for the gene coding for the HERV-W envelope, or with the expression plasmid coding for a hyperfusogenic variant of the amphotropic MLV envelope named ARless. These cells, named "producer cells", were then cocultured with human cells, termed "indicator cells", expressing the receptor for the

20

- 30 -

HERV-W envelope, and which also stably expressed either the envelope of GALV, the envelope of amphotropic MLV, or the envelope of RD114. The expression of these diverse envelope glycoproteins on these cells is capable of recognizing the corresponding receptors, of blocking them and therefore of decreasing their ability to interact with a retroviral envelope glycoprotein corresponding to them but expressed exogenously at the surface of the "producer" cells. Thus, if during the tests for fusion by coculturing a decrease is observed in the formation of syncytia for an indicator cell type which blocks one of these receptors compared to the parental indicator cell for which all of the three potential receptors are fully accessible, the nature of the receptor recognized by the envelope expressed on the producer cell may be deduced therefrom. After coculturing for two days, the cells are fixed and stained and the fusion indices determined. The results are given in table IV below.

Table IV

Envelope protein expressed in the producer cells	Envelope proteins expressed in the indicator cells			
	Control	MLV-A	GALV	RD114
Arless	+	-	+	+
HERV-W antisense	-	-	-	-
HERV-W	+	+	+	-

- signifies a lack of syncytia and + signifies the presence of syncytia

Control signifies that there is no envelope protein expressed in this cell.

These results make it possible to deduce that the envelope glycoprotein of HERV-W recognizes the hATB^o receptor for type D mammalian retroviruses. Specifically, although this envelope is fusogenic for the parental indicator cells or for the indicator cells expressing either the MLV-A envelope or the GALV

- 31 -

envelope, no syncytia are observed when the producer cells expressing the envelope glycoprotein of HERV-W are cocultured with the indicator cells expressing the RD114 envelope.

5

Example 4: Control of the fusogenic activity of Env HERV-W by its cytoplasmic component

The involvement of the cytoplasmic component of Env HERV-W in the fusogenic activity of this glycoprotein is demonstrated by the construction and characterization of the following recombinant glycoproteins:

W/CD46+, derived from human CD46, a factor protecting cells against complement and not involved in the formation of syncytia, comprising the ectodomain and the transmembrane domain of Env HERV-W (aa 1 to 469) fused to the cytoplasmic domain of CD46 (aa 335 to 369). This chimeric molecule is not fusogenic in a cell-cell fusion test (figure 5).

W/R+, derived from the envelope glycoprotein of the MLV-A (amphotropic murine leukemia virus) retrovirus, nonfusogenic when expressed independently of the other proteins of MLV-A, comprising the ectodomain and the transmembrane domain of Env HERV-W (aa 1 to 469) fused to the cytoplasmic domain of the envelope glycoprotein of the MLV-A retrovirus (aa 622 to 654). This chimeric molecule is not fusogenic in a cell-cell fusion test (figure 5).

RD/W, derived from the envelope glycoprotein of the RD114 feline endogenous retrovirus, nonfusogenic when expressed independently of the other proteins of RD114, comprising the cytoplasmic domain and the transmembrane domain of Env HERV-W (aa 448 to 538) fused to the ectodomain of the envelope glycoprotein of the RD114 retrovirus (aa 1 to 508). This chimeric molecule is fusogenic in a cell-cell fusion test (figure 5).

- 32 -

The attached figure 5 represents the scheme and characterization of the abovementioned Env HERV-W chimera and the results obtained in a cell-cell fusion test.

5

The ectodomain of Env HERV-W is defined by the polypeptide derived from the protein precursor containing amino acids (aa) 21 to 447; the transmembrane domain, aa 448 to 469 and the cytoplasmic component, aa 470 to 538 (Blond et al., (1999), Molecular characterization and placental expression of HERV-W, a new human endogenous retrovirus family. Journal of Virology. 73:1175-1185).

15 The ectodomain of CD46 is defined by the polypeptide derived from the protein precursor containing amino acids (aa) 35 to 312; the transmembrane domain, aa 313 to 334 and the cytoplasmic component, aa 335 to 369 (Yant et al., (1997), Identification of a cytoplasmic Tyr-X-X-Leu motif essential for down regulation of the human cell receptor CD46 in persistent measles virus infection. J. Virol. 71:766-770).

25 The ectodomain of Env MLV-A is defined by the polypeptide derived from the protein precursor containing amino acids (aa) 32 to 598; the transmembrane domain, aa 599 to 621 and the cytoplasmic component, aa 622 to 654 (Ott and Rein, (1990), Sequence analysis of amphotropic and 10A1 murine leukemia virus: close relationship to mink cell focus forming viruses. J. Virol. 64:757-766).

35 The ectodomain of Env RD114 is defined by the polypeptide derived from the protein precursor containing amino acids (aa) 18 to 508; the transmembrane domain, aa 509 to 530 and the cytoplasmic component, aa 531 to 564 (Cosset et al., (1995b), High titer packaging cells producing recombinant

- 33 -

retroviruses resistant to human serum. J. Virol.
69:7430-7436).

5 SU signifies surface subunit; TM transmembrane subunit;
SP signal peptide; tm transmembrane anchoring domain;
cyt cytoplasmic component; RBD receptor-binding domain;
PRR proline-rich region; C carboxy-terminal domain of
the SU.